Labeling oligonucleotides with ^{32}P ATP

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Wear gloves throughout and work in radiation area. Monitor area before and after use.

Mix the following in an eppendorf tube:
1. 0.5 microgram oligonucleotide dissolved in H2O.
2. 3 microliters 10x kinase buffer.
3. 2 microliters 32P ATP from ICN (>5000 ci/mmole).
4. H20 so that the final volume is 30 microliters.

Add 25 units T4 polynucleotide kinase and incubate 60 min at 37 deg.

If desired, double amounts of oligonucleotide and all other reagents to give 60 microliters total reaction volume. Purify phosphorylated oligo on a single spin column as described below. This will double the yield of radioactive primer.

Purify labeled Oligonucleotide away from unincorporated ATP

Currently, we use mini Quick Spin Oligo Columns (#1 814 397) from Roche to purify the labeled oligonucleotide.

Prepare the column according to the manufactuer's instructions by centrifugation of the resuspended matrix for 1 min @ 1000 x g.

Insert column into a new eppendorf tube and add oligo labeling reaction, adding slowly to center of column. Centrifuge 1000 x g for 4 min.

Recover purified labeled oligo. For most applications, add 70 microliters TE to the 30 microliters recovered for a total of 100 microliters.

Quantitate radioactive incorporation by counting 1 microliter of a 1/10 diluted sample. Expect between 20 -100 million cpm total.

10x Kinase Buffer
0.5 M Tris pH 7.6
0.1 M MgCl2
50 mM DTT